HEMOSTATIC ALTERATIONS FOLLOWING SEVERE DYSBARIC STRESS

by

Michael J. Jacey
Richard O. Madden
and
Donald V. Tappan

NAVAL SUBMARINE MEDICAL RESEARCH LABORATORY
REPORT NUMBER 773

Bureau of Medicine and Surgery, Navy Department
Research Work Unit Number M4306.02-5003BA9K.03

Reviewed and Approved by:

Charles F. Gell

Charles F. Gell, M.D., D.Sc. (Med)
SCIENTIFIC DIRECTOR
NavSubMedRschLab

Approved and Released by:

R. L. Sphar

R. L. Sphar, CDR MC USN
OFFICER IN CHARGE
NavSubMedRschLab

Approved for public release; distribution unlimited.

SUMMARY PAGE

THE PROBLEM

To evaluate the effects of dysbaric stress on the hemostatic (clotting) system in laboratory animals in order to better understand the effects of decompression injury in man.

FINDINGS

A series of hemostatic changes occurred in rats subjected to an inadequate decompression regimen and then observed over a three-day period. An early transient decline in whole blood clotting time, followed by a short-term elevation of plasma fibrinogen one day post-decompression, was detected. A persistent fever was evident by one day after surfacing. Also, platelet numbers were significantly reduced at about two days after experimental treatment. However, partial thromboplastin and prothrombin times were unchanged.

APPLICATION

These experiments indicate that very careful observation should be made over several days after decompression insult to detect symptoms of potentially serious damage which may have occurred even with a fairly normal subjective appearance of the injured individuals.

ADMINISTRATIVE INFORMATION

This investigation was conducted as part of Bureau of Medicine and Surgery Research Work Unit M4306.02-5003BA9K. The present report is number 3 on this work unit. It was submitted for review on 14 Jan 74, approved for publication on 12 February 1974 and designated as NavSubMedRschLab Report No. 773.

PUBLISHED BY THE NAVAL SUBMARINE MEDICAL RESEARCH LABORATORY

ABSTRACT

Hemostatic parameters were measured in the blood of mature Sprague-Dawley rats during a three day period following exposure to a compression-decompression schedule designed to produce severe dysbaric stress. The animals were compressed on air to a pressure equivalent to 300 feet of sea water for 30 minutes and stage decompression over a 42 minute interval. Acute decompression stress produced a transient decrease in clotting time. Circulating platelet population was unchanged during the early phase of recovery from severe decompression but it had declined significantly by two days post-surfacing. It then returned to control levels by the end of the observation period. Associated with this thrombocytopenic episode was a tendency toward platelet aggregation. Core temperature measurements indicated the presence of a chronic hyperthermic condition. A significant hyperfibrinogenemia had developed by one day post-dive with a normalization of fibrinogen content occurring during the following two days. No alterations in either prothrombin time or partial thromboplastin time were detected. This animal research has much significance in the biomedical aspects of human diver safety and health.

HEMOSTATIC ALTERATIONS FOLLOWING SEVERE DYSBARIC STRESS

INTRODUCTION

Decompression stress is characterized by alterations in hemostatic mechanisms. Central to this problem is a hemoconcentration[13] and a depletion of circulating platelets[1,2,14,16]. In a recent investigation, Jacey and associates[11] have described several aspects of the hypovolemia which may occur after completion of an inadequate decompression. It has been suggested by many workers that decompression sickness may initiate a condition similar to disseminated intravascular coagulation (DIC). Hardaway[6] has defined this condition as an acute, transient coagulation occurring in the flowing blood throughout the vascular tree. The numerous similarities between DIC and decompression sickness have been well documented by Holland[9].

Hypercoagulation following rapid decompression has been reported in dogs and rabbits by Aggazzotti and in mice, rats and rabbits by Barthelemy.[2] Philp and co-investigators[17] have demonstrated the existence of platelet thrombi surrounded by aggregated red cells in lungs of rats following severe decompression. In another study, Philp and his group also showed platelet depletion and hemoconcentration in rats exposed to a severe decompression schedule[16].

While considerable documentation exists concerning the changes in hemostatic parameters in acute dysbarism, little is known about the events occurring during a spontaneous recovery from exposure to an inadequate decompression. With this in mind, a study was undertaken to investigate hemostatic alterations during a period of recovery from severe dysbaric stress resulting from decompression insult.

For the purposes of this study, severe dysbaric stress was defined as that stress created by a decompression which is neither safe (100% survival) nor explosive (90-100% death within one hour post-surfacing)[12]. By these criteria, the 66% survival rate observed at one hour post-decompression would verify exposure to severe dysbaric stress. Stress-mediated leucocytic changes resulting from the use of this decompression routine has previously been reported[11].

METHODS AND MATERIALS

Mature male rats of the Sprague-Dawley strain weighing 530 $\pm$65 grams were pressurized in compressed air and decompressed according to a schedule designed to produce severe decompression stress[12]. The schedule entails pressurization to the equivalent of 300 feet of sea water (FSW) at 60 ft/min with a bottom stay of 25 min. Decompression is accomplished in two stages: 300 FSW to 60 FSW at 20 ft/min with a hold at 60 FSW for 15 min. and 60 FSW to the surface at 4 ft/min.

For each experiment, ten cages of six rats were placed in the outer lock of one of the Naval Submarine Medical Research Laboratory's man-rated chambers (volume 474 ft^3). Two cages of control animals were left next to the chamber

for the duration of each dive in order to be exposed to the noises of rushing air. Temperature profiles for this schedule[12] indicated that peaks of chamber temperature always occurred upon reaching 300 FSW (at 5 minutes from the surface) which were then promptly corrected and maintained by subsequent venting and decompression. Heat stress was minimal.

Following completion of the dive, all animals were held in clean cages for varying periods of time with adequate food and water available. All animals were weighed and handled daily. It should be emphasized that only control animals exposed to chamber noise were used.

At the appropriate time just prior to administration of anesthesia, core temperature in each animal was measured with a Yellow Springs Tele-Thermometer. The animals (experimental and control) were then injected intraperitoneally with 40 mg sodium pentobarbital/kg body weight. Blood was collected anaerobically in siliconized glass syringes from the abdominal aorta; and immediately thereafter, 0.5 ml of blood was dispensed into each of 3 siliconized 10 x 75 mm glass test tubes for the determination of clotting time according to a modified Lee-White procedure[19].

Without delay, 2 ml aliquots of whole blood were also transferred to glass tubes containing 0.2 ml 5% sodium citrate and 20 μl heparin (1000 units/cc), respectively. Blood and anticoagulants were mixed by several gentle inversions.

Prothrombin time and partial thromboplastin time were estimated using citrated blood. The remaining hemostatic parameters were measured utilizing heparinized blood with the exception of fibrinogen levels which were determined in heparinized plasma. Platelet and platelet aggregates were counted according to standard hematological methods[19,23]. Prothrombin time was measured with the Simplastin kit (General Diagnostics Corp.) while partial thromboplastin time was quantitated employing the Fibrosystem reagent package (Beckman Dickenson Corp.). Plasma fibrinogen estimations were performed according to a modification of the procedure of Rice and Muesse[19].

RESULTS

Although many investigators studying the effects of decompression stress have grouped depressurized animals according to the severity of symptoms, no attempt was made to classify our experimental animals on the basis of degree of injury. The following data presents all animals treated in each particular series.

The responses of several clotting parameters after severe decompression and a 3-day recovery period are shown in Table 1. A transient decrease in clotting time of almost two minutes was noted at one hour post-surfacing. Clotting times had normalized by one day post-decompression, remaining at this value for the duration of the observation period. No alteration in either prothrombin time or partial thromboplastin time was detected at any stage of the experiment.

While acute decompression stress had no discernible effects on the

Table 1. Clotting Parameters in Rat Blood Following Severe Decompression Stress

		Control	POST DECOMPRESSION 1 Hour	1 Day	2 Days	3 Days
Clotting Time Minutes	Mean	7.34	5.42*	7.33	7.39	6.90
	SEM	.49	.48	.59	.68	.41
	N	33	28	30	25	29
Platelet Count $\frac{\text{Cells} \times 10^3}{mm^3}$	Mean	878.3	826.1	876.5	771.4 *	897.7
	SEM	25.8	35.6	23.7	39.6	48.1
	N	58	35	57	38	39
Platelet Clumping Clumps per 1/5000 mm^3	Mean	7.4	8.5	6.9	10.4	8.7
	SEM	1.1	1.3	.8	1.3	1.1
	N	27	26	26	19	29
Prothrombin Time Seconds	Mean	13.1	13.0	12.6	12.9	13.1
	SEM	.2	.1	.2	.1	.1
	N	33	26	28	26	27
Partial Thromboplastin Time Seconds	Mean	14.1	14.3	14.2	14.4	13.8
	SEM	.2	.4	.2	.2	.2
	N	32	25	29	26	28

* Statistically significant at the 5% level or better.

circulating platelet population immediately following the dive, on the second day post-surfacing a marked decrease in cell number occurred. This was followed by a return to a normal count by the end of the observation. Coinciding with the significant drop in the circulating platelet population on the second day of the observation period, there was a tendency toward a higher number of platelet clumps, although not attaining statistical significance.

The results of studies of core temperature and fibrinogen levels following severe decompression are illustrated in Figure 1. Core temperature was markedly elevated at one day post-decompression with the hyperthermia persisting for the duration of the observation period. Fibrinogen concentrations rose significantly one day after the dive and declined gradually during the next two days of recovery from severe dysbaric stress.

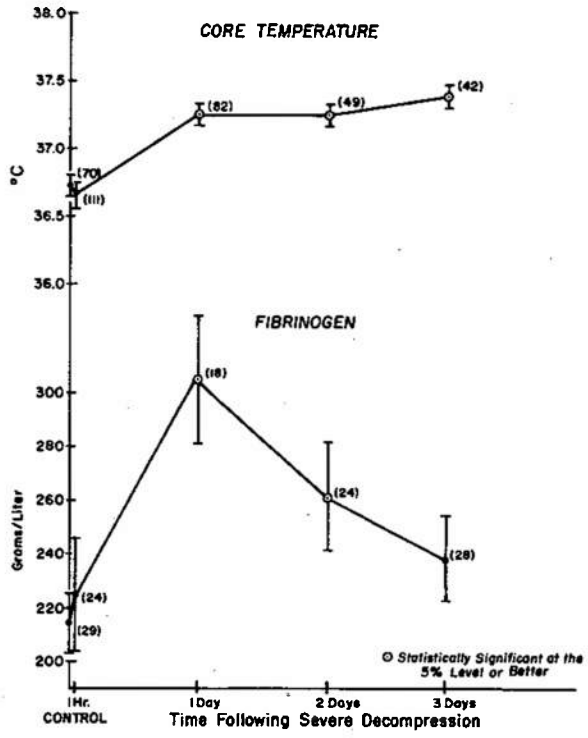

Fig. 1. Responses of Core Temperature and Plasma Fibrinogen Levels During Recovery from Dysbaric Stress in Rats. Numbers in ( ) equal number of animals in each group.

DISCUSSION

Several recent investigations in the field of dysbaric research have focused on the problems of hemostatic alterations following compression-decompression procedures. The Canadian group at Downsview[15] noted decreases in circulating platelets in human subjects with and without symptoms of dysbarism after a 3.5 hour decompression from an exposure to 10 ATA compressed air for 10 min. It was felt by these authors that the "possibility of hypercoagulation occurring post-decompression is realistic." A 37% reduction in platelet count together with a hypercoagulable state was also reported by Sicardi[20] in 3 divers surfacing from a 650 FSW dive. Bennett and Gray[3] detected a fall in platelets in 2 subjects who underwent a continuous 10.5 day decompression after a sojourn at 1500 FSW in a helium-oxygen atmosphere.

In contrast to the results of the experiments described above, Bonin and co-workers[4] collected evidence which indicated no alterations in hemostatic parameters in volunteers pressurized to 10 ATA on a $He-O_2-N_2$ mixture for 2 hours and then subjected to a borderline decompression regimen.

The significant factor in the above studies in contrast to our own, is that only the blood sample was collected per subject before the dive and after decompression, usually soon after reaching the surface. Animal experiments for studying hemostatic changes following compression-decompression have also generally followed the single pre-dive/post-dive sampling protocol.[15]

Philp and co-investigators[15] have discussed some possible factors affecting the changes in platelet population seen soon after decompression. These include: (1) decreased production, i.e., decreased megakarypoiesis; (2) active sequestration by organs such as the spleen; (3) increased utilization in the repair of vascular endothelium; and (4) the formation of platelet aggregates with subsequent trapping in the microcirculation. Based on the results of their hemostatic studies, these authors concluded that the last two factors were probably responsible for the depletion of circulating platelets seen soon after decompression. More recently,

Warren and associates[22] concluded that platelet adhesion to the interface combined with endothelial damage accounted for the drop in platelet counts seen in decompression situations, even when the mechanical blockage of the vasculature by gas bubbles is not significant. One can only speculate concerning the changes which might have been detected had the blood sampling been extended over a period of several days.

While a marked depression in clotting time was noted in our experiments at one hour post-surfacing, no acute changes in circulating platelet population were seen. However, by the second post-decompression day, a statistically significant decline in platelet number occurred which was normalized by the end of the observation period (day three). Although platelet aggregation was not altered statistically, a tendency toward an increased aggregation occurred concomitantly with the existence of the thrombocytopenic state. Similar results (i.e., a lack of early changes) were found by Martin and Nichols[14]. Moreover, they reported maximal platelet depletion 3 days after a compressed air dive to 100 FSW for 1 hour with a 122 minute decompression. In both of these experiments, cell depletion was not evident until long past the acute post-decompression stage. We attach considerable significance to the fact that similar results were obtained using either the very severe decompression schedule of our experiments or the mild schedule of Martin and Nichols[14].

Furnishing support for our observation, a shortening of coagulation time was noted by Aggazzotti[1] in 12 of 17 animals after exposure to pressures varying from 6 to 11 atmospheres. Alterations in coagulation in mice, rats and rabbits were also reported by Barthelemy[2] following rapid decompression. In addition, Hartmann[7] demonstrated changes in clotting factor parameters in dogs compressed and decompressed.

To facilitate discussion of possible hemostatic alterations resulting from dysbaric injury a schematic diagram depicting the interaction of the intrinsic and extrinsic pathways of coagulation is shown in Figure 2. Partial thromboplastin time, clinically, is a screening procedure for those factors involved in the intrinsic system of coagulation. These include those elements involved in the generation of plasma thromboplastin but not platelet function or Factor VII. One stage prothrombin time is the preferred test of the extrinsic pathway of coagulation including Factors I, II, VII, X, and V, but not Factor III, tissue thromboplastin[5,23].

Although fibrinogen (Factor I) was elevated at one day post-decompression, no changes were observed during the acute phase (1 hour) when the coagulation abnormality was demonstrated. It should be noted that hyperfibrinogenemia is not associated with coagulopathies[21,23].

Heyder and Tappan[8] in a series of separate experiments utilizing the same decompression table employed in this work, studied electrolyte dynamics following exposure of rats to severe dysbaric stress. Interestingly enough ionized calcium (Factor IV) was increased during the first hour and significantly decreased during the remainder of the period of observation. Currently,

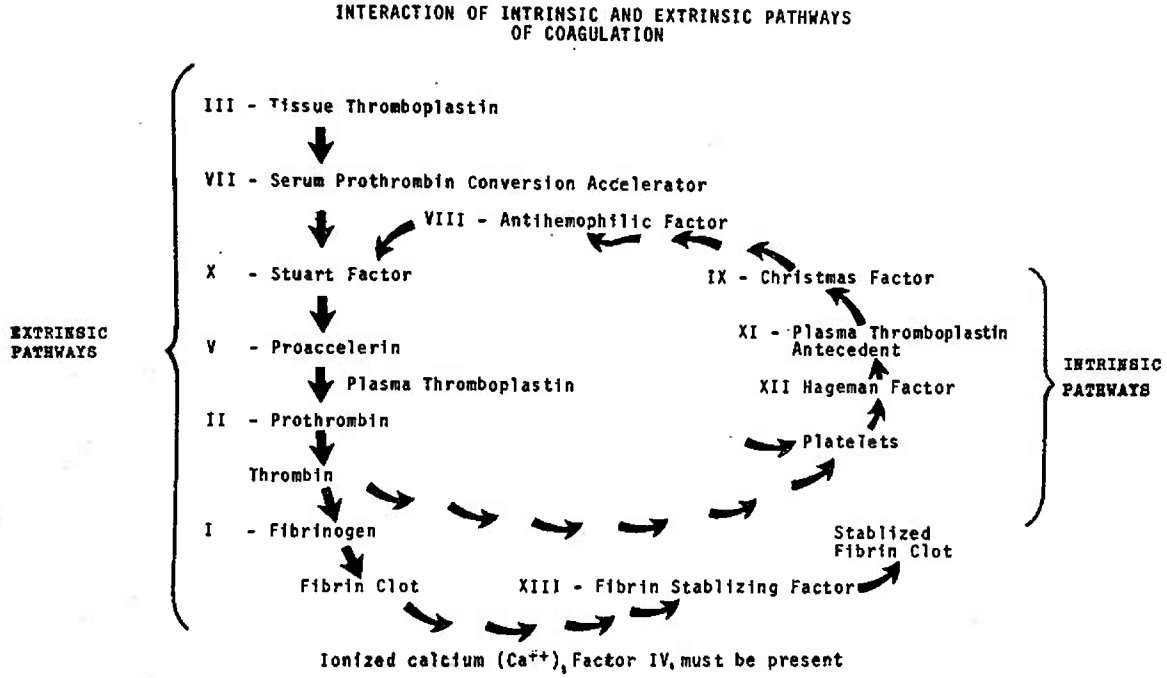

Fig. 2. Schematic Showing Coagulation Pathways.

hypercalcemia is believed to have no clinical significance in clotting defects[5,23]. The lack of change in both partial thromboplastin time and prothrombin time during the three day period of observation following severe decompression, especially during the first hour, coupled with the non-applicability of fibrinogen and calcium changes, would indicate that the origin of the clotting defects lies elsewhere than in those factors discussed above.

Although further studies are required to adequately define the site of the observed clotting defects, the acceleration of clotting time seen following trauma, as suggested by the work of Innes and Sevitt[10] may be due to the entry into the circulation of thromboplastin (Factor III) from injured tissue. Vascular tissue damage following severe decompression is well documented[17,22].

The picture that seems to be emerging from this series of studies designed to explore hemostatic alterations following exposure to inadequate decompression suggests a situation analogous in many ways to that reported by Innes and Sevitt[10] in injured patients. The sequelae outlined by these authors in patients that had undergone some sort of traumatization is as follows: (1) the first few hours are characterized by an acceleration of clotting time which is followed by a normalization of this parameter; (2) a thrombocytopenia occurs within one to three days; (3) plasma fibrinogen levels, usually rise within 24 hours. The similarities suggest a generalized response to non-specific injury generated by the inadequate decompression procedure utilized in these experiments. Hyperfibrinogenemia is well documented in trauma states, surgical as well as accidental, and seems

to result from overproduction by the liver[10,21]. The persistent hyperthermia recorded after the acute decompression period may also be taken as evidence of an inflammatory response to nonspecific tissue insult.

These findings demonstrate that inadequate decompression can result in an early clotting defect followed by the development of a latent syndrome of biochemical and hematologic abnormalities.

REFERENCES

1. Aggazzotti, A. Azione dell'aria compressa sugli animali. Viii. Il tempodi coagulazione del sangue. Boll. Soc. Ital. Biol. Sper. 8: 180-183, 1933.

2. Barthelemy, L. Blood coagulation and chemistry during experimental dives and the treatment of diving accidents with heparin. In: "Second Symposium on Underwater Physiology." Edited by: C.J. Lambertson and L.J. Greenbaum, Jr., p. 45-56, NRC, Washington, D. C., NRC Publication 1181, 1963.

3. Bennett, P. B. and S. P. Gray. Changes in human urine and blood chemistry during simulated O_2-helium dive to 1,500 feet. Aerosp. Med. 42: 868-874, 1971.

4. Bonin, B., P. W. Straub, R. Schibli, and A.A. Buhlmann. Blood coagulation during critical decompression following diving experiments with oxygen/helium. Aerosp. Med. 44: 508-512, 1973.

5. Handbook of Clinical Laboratory Data. The Chemical Rubber Co., H. C. Damm, editor. Cleveland, Ohio, pp. 329-345, 1965.

6. Hardaway, R. M. Syndromes of disseminated intravascular coagulation. Springfield, Ill. Charles C. Thomas, Publishers, 1966.

7. Hartmann, H. Blutgerinnungsuntersuchungen nach Dekompression. Inst. Z. angew. Physiol. einschl. arbeitphysiol. 18:439-443, 1961.

8. Heyder, E. and D.V. Tappan. Mineral and electrolyte responses following severe decompression stress. NAVSUBMEDRSCHLAB Report#743, 1973.

9. Holland, J.A. Discussion of disseminated intravascular coagulation in decompression sickness. NAVSUBMEDRSCHLAB Report #585, 1969.

10. Innes, D. and S. Sevitt. Coagulation and fibrinolysis in injured patients. J. Clin. Path. 17: 1-13, 1964.

11. Jacey, M.J., D.V. Tappan, and K. R. Ritzler. Hematologic responses to severe decompression stress. Aerospace Med 45: 417-421, 1974.

12. Jacey, M. J., D. V. Tappan, and J. J. Wojtowicz. A compression-decompression schedule for producing dysbaric stress in mature rats. NAVSUBMEDRSCHLAB Report#749, 1973.

13. Malette, W. G., J. B. Fitzgerald, and A.T.K. Cockett. Dysbarism. A review of thirty-five cases with suggestion for therapy. Aerosp. Med. 33: 1132-1139, 1962

14. Martin, K. J. and G. Nichols. Observation on platelet changes in man after simulated diving. Aerosp. Med. 43: 827-830, 1972.

15. Philp, R. B., K. N. Ackles, M. J. Inwoods, S. D. Livingstone, A. Achimastos, M. Binns-Smith, and M. W. Radomski. Changes in hemostatic system and in blood and urine chemistry of human subjects following decompression from a hyperbaric environment. Aerosp. Med. 43: 498-505, 1972.

16. Philp, R. B., C. W. Gowdey, and M. Prasad. Changes in blood lipid concentration and cell counts following decompression sickness in rats and the influence of dietary lipids. Canad. J. Physiol. Pharmacol. 45: 1047-1059, 1967.

17. Philp, R. B., M. J. Inwood, and B. A. Warren. Interaction between gas bubbles and components of the blood: Implications in decompression sickness. Aerosp. Med. 43: 946-953, 1972.

18. Practical Manual for Clinical Laboratory Procedures. The Chemical Rubber Co., H. C. Damm, editor. Cleveland, Ohio, 1965.

19. Rice, E. W. and D. E. R. Muesse. Clinical nephelometry: New accurate, heat-precipitation nephelometric method for rapid determination of plasma fibrinogen. Clin. Chem. 18: 73-75, 1972.

20. Sicardi, F. La coagulation au cours de la plongee profonde. Bull. Medsubhyp. 4: 15-16, 1970.

21. Stahl, M. Fibrinogenopathie. Acta. Haematol. Pol. 3: 93-99, 1972.

22. Warren, B. A., R. B. Philp, and M. J. Inwood. The ultrastructural morphology of air embolism: Platelet adhesion to the interface and endothelial damage. Brit. J. Exp. Pathol. 54(2): 163-172, 1973.

23. Wintrobe, M. M. Clinical Hematology. Phildelphia: Lea and Febiger, 1961.

UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)*
NAVAL SUBMARINE MEDICAL RESEARCH LABORATORY, Naval Submarine Medical Center

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
HEMOSTATIC ALTERATIONS FOLLOWING SEVERE DYSBARIC STRESS

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*
Interim report

5. AUTHOR(S) *(First name, middle initial, last name)*
Michael J. JACEY, Richard O. MADDEN and Donald V. TAPPAN

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
12 February 1974	8	22

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO.	NSMRL Report Number 773
c. M4306.02-5003BA9K.03	9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)*
d.	

10. DISTRIBUTION STATEMENT
Approved for public release; distribution unlimited

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Naval Submarine Medical Rsch Laboratory Box 900 Naval Submarine Base Groton, Connecticut 06340

13. ABSTRACT

Hemostatic parameters were measured in the blood of mature Sprague Dawley rats during a three-day period following exposure to a compression-decompression schedule designed to produce severe dysbaric stress. The animals were compressed on air to 300 feet of sea water for 30 minutes and stage decompressed over a 42-minute interval. Acute decompression stress produced a transient decrease in clotting time. Circulating platelet population was unchanged during the early phase of recovery from severe decompression but significantly declined by two days post-surfacing and then returned to control levels by the end of the observation period. Associated with this thrombocytopenic episode was a tendency toward platelet aggregation. Core temperature measurements indicated the presence of a chronic hyperthermic condition. A significant hyperfibrinogenemia developed by one day post-dive with a normalization of fibrinogen content occurring during the following two days. No alterations in either prothrombin time or partial thromboplastic time were detected. This animal research has much significance in the biomedical aspects of human diver safety and health.

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
dysbaric stress						
clotting defect						
hyperthermia						
hyperfibrinogenemia						
thrombocytopenia						